## REMARKS

Claims 9-12, 20-22, 24-26, 28-32, and 34-36 are pending in the application, with claim 9 being currently amended.

Applicants' undersigned representative appreciates the courtesies extended by the Examiner during the telephonic interview held on November 2, 2010. During that interview, independent claim 9, the rejection thereof, and proposed substitute claims therefore, were discussed. At which time, the Examiner offered a number of suggestions for amending claim 9, which have been heavily adopted herein, to clarify the claimed invention and differentiate it from the cited art to overcome the present rejections. While no agreement was reached at that time, Applicants' undersigned representative found the Examiner's comments and suggestions to be highly beneficial in preparation of the present response.

To that end, claim 9, which is the only independent claim, has been amended to more clearly define over the art of record. In particular, claim 9 now recites a process for producing lactoperoxidase that comprises, in part, a step (1) for bringing one or more milk materials, which include lactoperoxidase, into contact with a cation exchanger to effect adsorption of the lactoperoxidase; a step (2) for washing the cation exchanger after said adsorption of the lactoperoxidase; a step (3) for bringing said washed cation exchanger into contact with a leaching solvent which elutes the lactoperoxidase, wherein an ionic strength of the leaching solvent is 0.07 to 0.3, to obtain a leaching solution, which includes the lactoperoxidase; a step (4) for concentrating said leaching solution by passing a portion of said leaching solution through an ultrafiltration membrane so that the protein content in the retentate becomes 0.9 to 15%, and wherein proteins other than the lactoperoxidase precipitate out in the retentate; and a

step (5) for obtaining a lactoperoxidase solution by removing the precipitate from the retentate. Support for the amendments can be readily found throughout the application.

**35 U.S.C. §112 rejections**

In the Official Action, previously pending claim 9 stands rejected under 35 U.S.C. 112, second paragraph, as being indefinite. In rejecting claim 9, the Examiner states that it is unclear what Applicants intend to claim with the phrase "the precipitation is not re-dissolved in purified water". See Official Action at Page 2.

Applicants have removed the offending phrase from claim 9 thereby rendering the claim definite and the rejection now moot. Accordingly, the rejection is overcome and must be withdrawn.

**35 U.S.C. §103 rejections**

In the Official Action, Examiner continues to reject previously pending claims 9-12, 20-22, 24-26, 29-32, and 34-36 under 35 U.S.C. 103(a) as being unpatentable over Uchida U.S. Patent No. 5,516,675 ("Uchida"), Burling U.S. Patent No. 5,149,647 ("Burling"), Kussendrager U.S. Patent Nos. 6,010,698 and 5,596,082 ("the Kussendrager '698 patent" and "the Kussendrager '082 patent", respectively) (collectively, "the Kussendrager patents"), Souppe FR 2841747 (as evidenced by U.S. Patent No. 7,247,331) ("Souppe"), and Lihme U.S. Patent No. 5,780,593 ("Lihme"). *See* Official Action at Pages 3-7. Applicants respectfully disagree with the present rejections, particularly in view of independent claim 9 as currently amended.

Briefly by way of background, in the present invention, an eluting treatment of a cation exchanger, in which a specific leaching solution having specific ionic strength is used, and a concentration treatment, in which a concentration of protein to a specific range is conducted by an ultrafiltration method, are combined. Due to the combination, unexpected excellent effects

are achieved such that, as shown in step (4), when a leaching solution, which includes lactoperoxidase, is concentrated through an ultrafiltration membrane to achieve 0.9 to 15% of a total protein content in the retentate, a precipitate of proteins other than lactoperoxidase is formed in the retentate. And while the precipitate of proteins other than lactoperoxidase is formed as a solid in the concentrated leaching solution, lactoperoxidase is dissolved in the concentrated leaching solution, i.e., the retenate. Furthermore, the precipitate formed in step (4) can be easily collected and removed in step (5). In this way, the ultrafiltration treatment of the present invention makes it possible to generate a precipitate of protein impurities, which are different from lactoperoxidase, in a concentrated leaching solution. Further to that end, after the ultrafiltration treatment, it is possible to obtain a solution including lactoperoxidase at a high concentration merely by removing the precipitate (precipitate of proteins other than lactoperoxidase) from the retentate. After removing the precipitate, it is also possible to obtain high purity lactoperoxidase by drying the solution from which the precipitate has been removed. The excellent characteristic of the present invention enables easy performance of a desalting treatment. See, e.g., page 21, lines 16-18 of the present specification.

Applicants now specifically address the present §103 rejections. Even assuming *arguendo* that one skilled in the art would combine Uchida, Burling, the Kussendrager patents, Souppe, and Lihme, which we assert one would not, the combination still fails to make obvious Applicant's process for producing lactoperoxidase, as now recited in claim 9. Indeed, to establish *prima facie* obviousness of a claimed invention, it is certainly well established that all the claim limitations must be taught or suggested by the prior art. *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974); *See also* MPEP §2143.03 (citing *In re Wilson*, 424 F.2d 1382, 1385, 165 USPQ 494, 496 (CCPA 1970))(To establish *prima facie* obviousness of a claimed invention,

it is certainly well established that "all words in a claim must be considered when judging the patentability of that claim against the prior art or suggested by the prior art." (emphasis added)).

In the instant case, Applicants respectfully submit that the Examiner has failed to establish a *prima facie* case of obviousness for the reasons that follow.

Again, claim 9 now requires a step (4) for concentrating the leaching solution by passing a portion of said leaching solution through an ultrafiltration membrane so that the protein content in said concentrated leaching solution, which is retentate, becomes 0.9 to 15%, and wherein proteins other than the lactoperoxidase precipitate out in the retentate, and a step (5) for obtaining a lactoperoxidase solution by removing the precipitate the retentate.

Upon review of Uchida, this reference discloses that an ultrafiltration method is performed but there is no disclosure or suggestion that the ultrafiltration method is used to separate, via precipitation, lactoperoxidase, as a soluble fraction, from other proteins, as an insoluble fraction by concentrating the total protein content in the retentate of the leaching solution to 0.9% to 15%, and then removing the precipitate from the retentate, which includes the soluble lactoperoxidase, as is now required by claim 9. See col. 6, lines 12-18. In support thereof, Uchida states only the following:

> The isolation and purification of lactoperoxidase, secretory component, and lactoferrin by the present invention, requires no repeated chromatographic isolation and purification procedures and can be performed by simple methods. That is, the present invention gave lactoperoxidase, secretory component, and lactoferrin at purity of 80% or over in a single chromatographic treatment. Additional treatment with an ultrafiltration eliminates a small amount of low molecular weight fraction and provides lactoperoxidase, secretory component, and lactoferrin at purity of 85% or over. The simplified process provides not only highly pure lactoperoxidase, secretory component, and lactoferrin at high yield but also reduces production cost. The products can be used for foods and pharmaceuticals for the treatment and prevention of

infectious diseases and anemia." Emphasis added. Col. 8, lines 5-18.

To that end, the ultrafiltration methods disclosed in Uchida separate and remove components based on molecular weight by passing low molecular weight proteins through the ultrafiltration membrane. In stark contrast, in the present invention, when lactoperoxidase adsorbed into the cation exchanger is eluted into the leaching solvent, the lactoperoxidase is obtained in a mixture with other protein fractions (impurities). Then, when concentration is performed using an ultrafiltration membrane, the other protein fractions (impurities) are selectively isolated via precipitation by concentrating the protein content to 0.9% to 15% in the retentate wherein proteins other than the lactoperoxidase precipitate out in the retentate. Then, the precipitate is removed from the renentate, which includes the soluble lactoperoxidase. Accordingly, separation of the lactoperoxidase from other protein fractions is based on differences in solubility, and not at all on molecular weight. As a result, high purity lactoperoxidase can be obtained. See page 8, lines 5-7 of the present specification.

The isolation of the lactoperoxidase in the present invention is carried out based on the characteristics that solubility of lactoperoxidase is different from that of proteins other than lactoperoxidase. Within a concentrated solution generated by the ultrafiltration (that is, within a solution which has not passed through an ultrafiltration membrane and exists as a retentate), lactoperoxidase (a soluble fraction) and proteins other than lactoperoxidase (a precipitation fraction) are separated. And such a separation method is neither disclosed nor suggested by Uchida.

Furthermore, after ultrafiltration, Uchida fails to take note of the solubility of the components included in a concentrated solution. For example, in Example 1 of Uchida, after

concentration and desalting are conducted with an ultrafiltration membrane having a molecular weight cut-off of 10,000, lyophilization is simply performed to obtain 12 g of lactoperoxidase at a purity of 90%, 8 g of secretory component at a purity of 85%, and 17 g of lactoferrin at a purity of 95%. Based on such a disclosure, a person skilled in the art cannot come up with formation of a precipitate of proteins other than lactoperoxidase in accordance with the solubility difference of components in the solution. In addition, a person skilled in the art is unable to discern from Uchida that, when a protein included in a leaching solution concentrated with an ultrafiltration membrane is controlled to have a specific concentration, lactoperoxidase and proteins other than lactoperoxidase can be separated due to the solubility difference thereof. In this way, the present invention provides unexpected excellent effects which have not been achieved, and non-obviousness of the present invention, as embodied by independent claim 9, is evident. And the remaining references cited by Examiner fail to cure the aforementioned deficiencies.

Regarding the remaining references, i.e., Burling, the Kussendrager patents, Souppe, and Lihme, the present invention likewise provides unexpected excellent effects which have not been disclosed therein. In the present invention, due to the solubility differences between lactoperoxidase and proteins other than lactoperoxidase, a precipitate forms in the retentate of the leaching solution during ultrafiltration, yet, lactoperoxidase remains dissolved in the solution. There are no references that take note of the solubility of components in a concentrated solution obtained after ultrafiltration. Accordingly, a person skilled in the art cannot arrive at the generation of a precipitate of proteins other than lactoperoxidase using the solubility differences. Indeed, a person skilled in the art cannot arrive at the separation of lactoperoxidase and proteins other than lactoperoxidase, as is required by claim 9, since the remaining cited references fail to disclose or suggest either of controlling a protein content of a

leaching solution concentrated by an ultrafiltration to a specific range or using the solubility differences between lactoperoxidase and proteins other than lactoperoxidase. To that end, Applicants now address each of the remaining references in more detail below, in turn.

With respect to Burling, this reference discloses that milk serum is first treated by microfiltration for removal of residues of fat and protein aggregate particles. The microfiltered milk serum is then passed at a high rate through a column packed with a strong cation exchanger, which selectively adsorbs lactoperoxidase and lactoferrin. The elution of the ion exchange mass is started by washing the milk serum out of the column with a buffer. Subsequently, impurities, if any, are eluted with a buffer solution containing a weak saline solution. After this preparatory elution, the desired proteins are selectively eluted with buffer solutions containing saline solutions, at different concentrations. Thus, the elution of lactoperoxidase is performed at a salt concentration in the range of 0.10-0.4 M, and of lactoferrin at a salt concentration within 0.5-2 M. After this treatment, the proteins concerned have been concentrated about 500 times. The pure protein fractions are collected, and then a further concentration is preferably effected by ultrafiltration followed by desalination and freeze-drying so as to obtain a commercial product consisting of about 90% pure protein fractions. See col. 3, line 47 to col. 4, line 17.

Based on the above, it is clear that Burling fails to disclose or suggest an ultrafiltration method that is used to separate, via precipitation, lactoperoxidase, as a soluble fraction, from other proteins, as an insoluble fraction by concentrating the total protein content in the retentate of a leaching solution to 0.9% to 15%, and then removing the precipitate from the retentate, which includes the soluble lactoperoxidase, as is required by claim 9.

With respect to the Kussendrager '698 patent, this reference relates only to a process for recovering growth factors, or a composition containing one or more growth factors,

from milk or a milk derivative, and further discloses only that:

> Lactoferrin and lactoperoxidase can be eminently recovered from milk or milk products on an industrial scale. U.S. Pat. No. 5,596,082 discloses a process in which lactoferrin and lactoperoxidase are adsorbed to a cation exchanger by passing milk or a milk product at a high superficial velocity (more than 500 cm per hour) and at a high liquid load (100-600 bed volumes per hour) over the cation exchanger and then eluting the cation exchanger with a number of salt solutions of different concentrations. Thus a lactoferrin-containing fraction and a lactoperoxidase-containing fraction are obtained, which can be further treated in a conventional manner. Col. 1, lines 15-25.

Further concerning the Kussendrager '698 patent, the reference also discloses that the cation exchanger to which the components from the milk or milk derivative are adsorbed can be any conventional cation exchanger in this field of the art. Col. 3, lines 23-25. Also, elution with a solution of a low NaCl concentration, for instance of between 0.15 and 0.25 molar, yields a fraction containing substantially lactoperoxidase. Col. 4, lines 1-3. And the fractions obtained, containing substantially lactoferrin and lactoperoxidase respectively, can be further treated according to conventional methods. These methods can comprise the steps of desalting, concentration, removing bacteria, and drying. Col. 4, lines 16-18.

With respect to the Kussendrager '082 patent, this reference is directed to a process for isolating the metalloprotein lactoferrin and the enzyme lactoperoxidase from milk and milk products on an industrial scale. The process includes the steps of adsorbing these proteins to a cation exchanger by passing milk or the milk derivatives over the cation exchanger at a high superficial velocity (more than 500 cm per hour) and at a high liquid load (100-600 bed volumes per hour); eluting these proteins, separately or simultaneously, by elution with one or more salt solutions, so as to form one or more eluates; and optionally followed by drying of the eluates. The binding capacity of the ion exchanger is more than 10 g lactoperoxidase and more

than 10 g lactoferrin per liter bed volume, and wherein more than 80% of the lactoperoxidase and lactoferrin is extracted.

Notably, based on the above, neither the Kussendrager '082 patent nor the Kussendrager '698 patent disclose or suggest an ultrafiltration method that is used to separate, via precipitation, lactoperoxidase, as a soluble fraction, from other proteins, as an insoluble fraction by concentrating the total protein content in the retentate of a leaching solution to 0.9% to 15%, and then removing the precipitate from the retentate, which includes the soluble lactoperoxidase, as is required by claim 9.

With respect to Souppe, this reference discloses a method including the following steps (a) to (d) wherein milk proteins are isolated from milk or from whey:

a) milk or the whey is sterilized and defatted;

b) the milk fraction derived from step a) is passed over a cation-exchange resin conditioned in an elution column;

c) the fraction retained on the resin is eluted with an aqueous salt solution;

d) the eluate resulting from step c) is desalted and sterilized preferably by ultrafiltration and diafiltration, and then sterilized preferably by microfiltration. Col. 2, lines 44-54.

Based on the above, Applicants submit that Souppe fails to disclose or suggest an ultrafiltration method that is used to separate, via precipitation, lactoperoxidase, as a soluble fraction, from other proteins, as an insoluble fraction by concentrating the total protein content in the retentate of a leaching solution to 0.9% to 15%, and then removing the precipitate from the retentate, which includes the soluble lactoperoxidase, as is required by claim 9.

Finally, with respect to Lihme, this reference discloses a method for separating

biological molecules such as protein, e.g., lactoperoxidase, and peptide from a medium including such biological molecules by ion exchange. Lihme further discloses that it was it was observed that ion exchangers of the weak type, e.g. non-quarternary amine based anion exchangers such as diethylaminoethyl (DEAE)-based ion exchangers and carboxylic acid based cation exchangers, can be eluted so that the neutralization of the ion exchanging (charged) groups on the ion exchange resin provide an uncharged resin. Further, it was observed that a gradual neutralization of the charged ion exchanging groups functioning as a buffer substance ensured that the bound and released biomolecule was kept in a medium having a low salt content and non-extreme pH-values which were acceptable to the eluted biomolecules. This gradual neutralization is against the general teaching of ion exchange chromatography, e.g. see "Ion Exchange Chromatography--Principles and Methods", PHARMACIA FINE CHEMICALS, Uppsala, Sweden, March 1980, where anion exchangers are taught to be eluted with a decreasing pH gradient and cation exchangers are taught to be eluted with an increasing pH gradient. Further, this ensures that no buffer substances are required in the eluant, because the buffering effect is associated with the ion exchanging groups of the ion exchanger. Abstract and col. 4, lines 12-34.

Notably, Lihme fails to disclose or suggest an ultrafiltration method that is used to separate, via precipitation, lactoperoxidase, as a soluble fraction, from other proteins, as an insoluble fraction by concentrating the total protein content in the retentate of a leaching solution to 0.9% to 15%, and then removing the precipitate from the retentate, which includes the soluble lactoperoxidase, as is required by claim 9

In view of all of the above, when combined, Uchida, Burling, the Kussendrager patents, Souppe, and Lihme fail to provide all of the elements of Applicants' claimed process for producing lactoperoxidase. That is, Examiner has not established a *prima facie* case of

obviousness based on these disclosures. In particular, the combination fails to disclose or suggest an ultrafiltration method that is used to separate, via precipitation, lactoperoxidase, as a soluble fraction, from other proteins, as an insoluble fraction by concentrating the total protein content in the retentate of a leaching solution to 0.9% to 15%, and then removing the precipitate from the retentate, which includes the soluble lactoperoxidase.

Accordingly, the rejections are overcome and must be withdrawn. Applicants, thus, respectfully submit that independent claim 9, along with its dependent claims, is allowable over the cited references.

**Conclusion**

As a result of the remarks given herein, Applicants submit that the rejection of the pending claims has been overcome. Therefore, Applicants respectfully submit that this case is in condition for allowance and request allowance of the pending claims.

If Examiner believes any detailed language of the claims requires further discussion, Examiner is respectfully asked to telephone the undersigned attorney so that the matter may be promptly resolved. Applicants also have submitted all fees believed to be necessary herewith. Should any additional fees or surcharges be deemed necessary, Examiner has authorization to charge fees or credit any overpayment to Deposit Account No. 23-3000.

Respectfully submitted,
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